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Original article

The role of mobile phones as a possible pathway for pathogen movement, a cross-sectional microbial analysis

Lotti Tajouri [a,b,e,j,*,1], Mariana Campos [b,i], Matthew Olsen [a], Anna Lohning [a], Peter Jones [a], Susan Moloney [a,h], Keith Grimwood [g,h], Hassan Ugail [f], Bassam Mahboub [d], Hamad Alawar [c], Simon McKirdy [b,1], Rashed Alghafri [a,b,c,e,j,1]

[a] Faculty of Health Sciences and Medicine, Bond University, Robina, QLD, Australia
[b] Harry Butler Institute, Murdoch University, Murdoch, WA, 6150, Australia
[c] General Department of Forensic Science and Criminology, Dubai Police, Dubai, United Arab Emirates
[d] Dubai Health Authority, Dubai, United Arab Emirates
[e] Dubai Future Council on Community Security, Dubai, United Arab Emirates
[f] Centre for Visual Computing, University of Bradford, Bradford, United Kingdom
[g] Griffith University and Gold Coast Health, Southport, QLD, Australia
[h] Department of Paediatrics, Gold Coast University Hospital, Southport, Australia
[i] CSIRO Health & Biosecurity, CSIRO Land & Water, Australia
[j] Dubai Police Scientists Council, Dubai Police, Dubai, United Arab Emirates

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ABSTRACT

Introduction: Mobile phones are used the world over, including in healthcare settings. This study aimed to investigate the viable microbial colonisation of mobile phones used by healthcare personnel.
Methods: Swabs collected on the same day from 30 mobile phones belonging to healthcare workers from three separate paediatric wards of an Australian hospital were cultured on five types of agar plate, then colonies from each phone were pooled, extracted and sequenced by shotgun metagenomics. Questionnaires completed by staff whose phones were sampled assisted in the analysis and interpretation of results.
Results and discussion: All phones sampled cultured viable bacteria. Overall, 399 bacterial operational taxonomic units were identified from 30 phones, with 1432 cumulative hits. Among these were 58 recognised human pathogenic and commensal bacteria (37 Gram-negative, 21 Gram-positive). The total number of virulence factor genes detected was 347, with 1258 cumulative hits. Antibiotic resistance genes (ARGs) were detected on all sampled phones and overall, 133 ARGs were detected with 520 cumulative hits. The most important classes of ARGs detected encoded resistance to beta-lactam, aminoglycoside and macrolide antibiotics and efflux pump mediated resistance mechanisms.
Conclusion: Mobile phones carry viable bacterial pathogens and may act as fomites by contaminating the hands of their users and indirectly providing a transmission pathway for hospital-acquired infections and dissemination of antibiotic resistance. Further research is needed, but meanwhile adding touching mobile phones to the five moments of hand hygiene is a simple infection control strategy worth considering in hospital and community settings. Additionally, the implementation of practical and effective guidelines to decontaminate mobile phone devices would likely be beneficial to the hospital population and community at large.

1. Introduction

Mobile phones have transformed healthcare allowing instant communication and clinical resource utilisation. The World Health Organization has defined mobile health (mHealth) as "… medical and public health practice supported by mobile devices, such as mobile phones …" [1–3]. Both in community and healthcare settings, the use of mobile phones is universal [4–8].

* Corresponding author. Bond University, Robina, Australia.
E-mail address: ltajouri@bond.edu.au (L. Tajouri).
[1] Chief Investigators.

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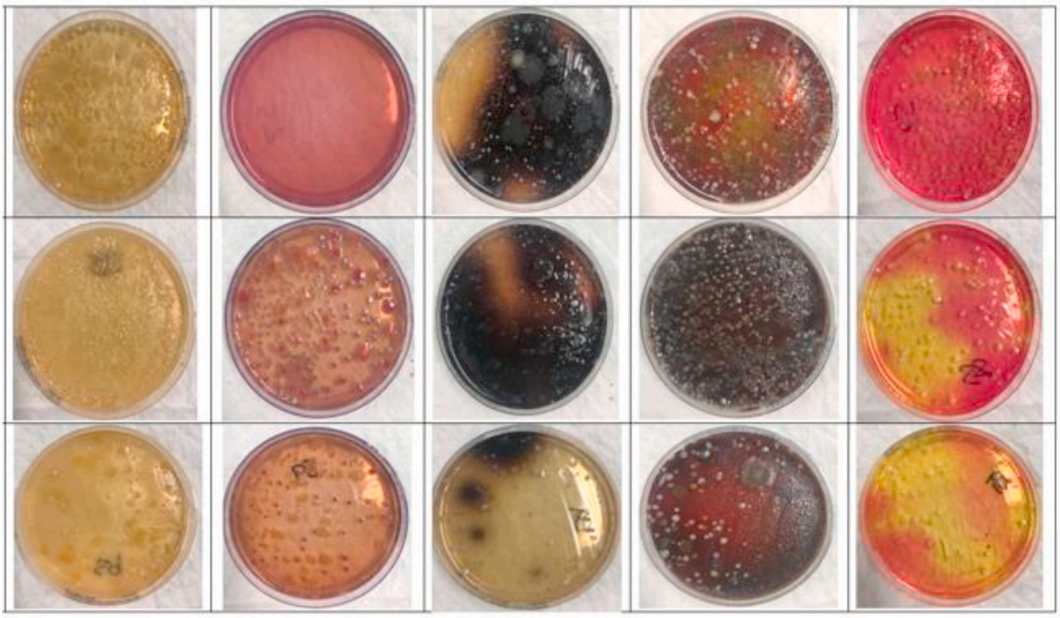

Fig. 1. Examples of agar plates for three phones from the Paediatric Emergency Department. Each row consists of five petri agar plates (Nutrient agar, MacConkey agar, Bile esculin agar, horse blood agar and Mannitol Salt agar) initially inoculated from a unique phone swab.

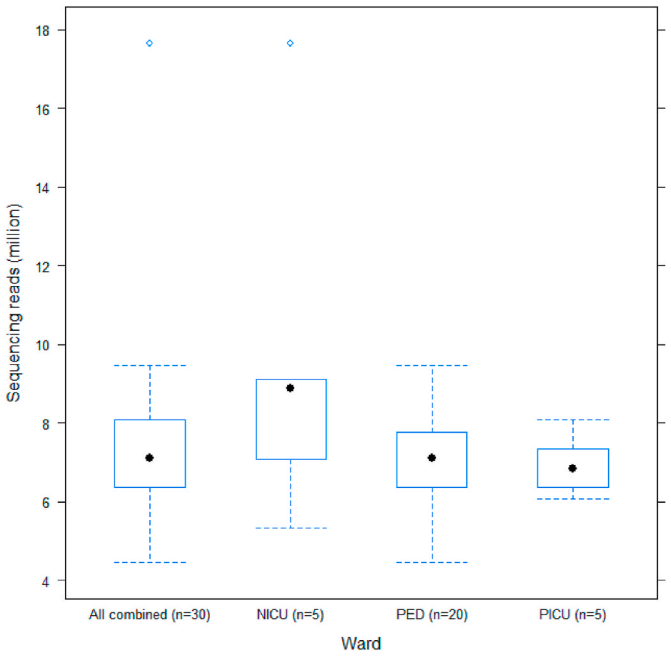

Fig. 2. Sequencing reads found in the sampled phones per ward. NICU=Neonatal Intensive Care Unit, PED=Paediatric Emergency Department, PICU=Paediatric Intensive Care Unit. No significant differences were observed (P = 0.149).

People treated in hospitals are vulnerable to hospital-acquired infections (HAI), which pose a major health threat worldwide as a leading cause of morbidity and mortality. It was estimated that, from 2010 to 2016, Australian hospitals had approximately 165,000 HAIs per year [9], while US hospitals had 687,200 HAIs in 2015 [10]. The costs associated with treating HAIs, in 2009, were estimated at $AUD942 million per year in Australia [11] and estimates for the United States ranged from $USD28 billion to $USD45 billion [12]. One of the main drivers for the high cost of HAIs is the global increase in antimicrobial resistance observed in pathogenic bacteria [13]. It has been estimated that one-third of these infections could be prevented by adhering to standard infection control guidelines [14].

A recent systematic review [15] identified mobile phones as potential 'Trojan horses', due to contamination with various microbes, including bacteria, fungi and viruses. It also found that the organisms detected on the phones of healthcare workers had higher prevalence of antimicrobial resistance than the control groups [15]. Multiple-drug resistant organisms (MROs) have also been found on other touchscreen devices outside of the hospital environment [16]. A study in India showed approximately 10% of isolates from automatic teller machines had antibiotic resistance [17], and in Arizona, USA, MROs were found on touch screens of self-checkouts at the supermarket [16]. Additionally, a recent study of phones belonging to butchers, cooks, farmers, students, diary employees and health workers reported a high degree of microbial contamination [18]. It has also been suggested that food handlers using phones while working may lead to foodborne infections [18].

A recent study showed that 77.8% of swab samples taken from mobile phones of known positive COVID-19 individuals in 11 quarantine and biocontainment units were positive for SARS-CoV-2 RNA [19]. A second study in a COVID-19 isolation ward subdivided into three zones (contaminated, semi contaminated and clean) using disinfecting procedures showed that in both the "clean 'and 'semi contaminated' zones physician's phones were positive for SARS-CoV-2 RNA [20].

The importance of mobile phones as fomites is threefold. Firstly, they are omnipresent in the community, with an estimated 5.16 billion mobile phone users globally in 2020 [21]. Secondly, mobile phones are objects in close contact with our hands and face with high touch frequency. A study in an office setting registered an average of 26.8 hand touches per hour on mobile phones [22]. Thirdly, multiple surveys have shown that phones are rarely or never cleaned [14,23,24], even though there is evidence that regular cleaning of mobile phones reduces the contamination rate in the short-term [14,25–27].

Despite a growing number of studies highlighting mobile phones as microbe contaminated platforms [15], particularly with MROs in healthcare settings [28], it is unlikely that the full understanding of the extent of contamination is known. Previous reported studies have utilised swab-culture-morphological and biochemical identification methodologies, which have presented two bottlenecks: (1) the culture media

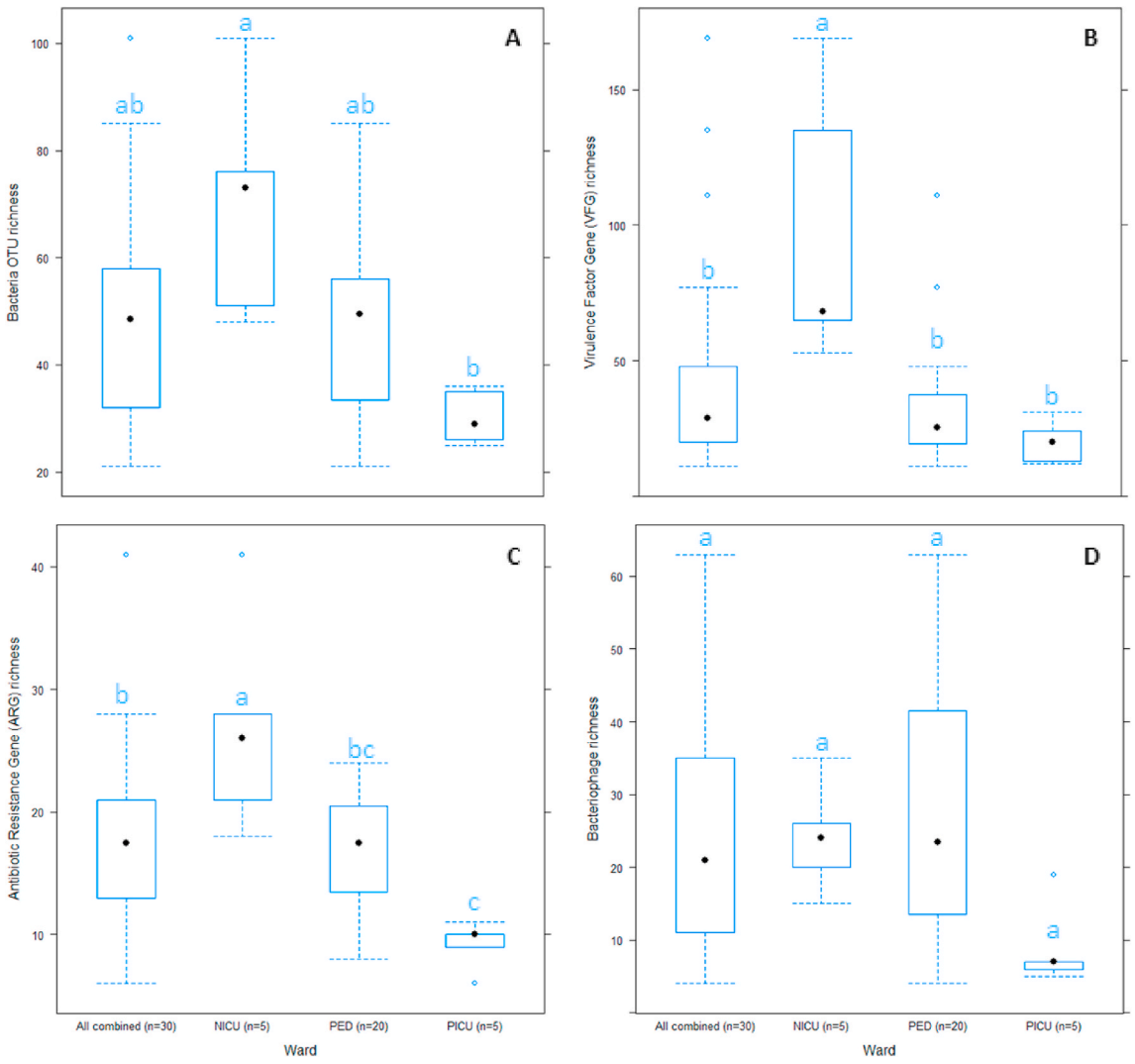

Fig. 3. Boxplots of richness of (A) bacterial operational taxonomic units (OTU), (B) virulence factor genes, (C) antibiotic resistance genes and (D) bacteriophages found in all sampled mobile phones and ward subsamples. Letters above the boxplot indicate significant difference calculated with Tukey's HSD test from analyses of variance. Calculated P values were: 0.0114 (a), 0.00154 (b), 0.000639 (c), and 0.116 (d).

does not allow for all organisms to grow; and (2) the identification of organisms is often limited in taxonomic resolution. A study comparing swab-culture morphological identification to culture-independent swab-PCR identification to the genus level highlighted that there are limitations with the first method [29]. However, questions remain over omitting the culture stage, as this step allows the researcher to confirm that the organisms detected were viable and thus potentially infectious [29]. Other studies have used instead whole genome sequencing of a few isolated agar based cultured bacteria identified initially by means of 16s RNA sequencing [30].

Much effort is undertaken by scientists and healthcare personnel to reduce community and HAIs, and multiple calls have been made to develop standardised protocols for regular phone cleaning by healthcare staff and patients [28,31,32]. Unfortunately, to our knowledge, no protocols or functional pan-systemic implementation have been agreed upon or deployed nationally or internationally. Furthermore, such protocols are unlikely to be effective without strict attention to hand hygiene.

The aim of this study was to characterise viable microbes on mobile phones from healthcare workers to the narrowest taxonomic unit through the swab-culture-next generation sequencing technique. The secondary aim was to look at the occurrence rate of virulence factor genes (VFGs) and antimicrobial resistance genes (ARGs).

2. Methods

Mobile phone samples and associated user surveys were collected from staff members working in the Paediatric Emergency Department (PED), Neonatal Intensive Care unit (NICU) and Paediatric Intensive Care Unit (PICU) at the Gold Coast University Hospital, South East Queensland, Australia.

Sampling was undertaken with phones swabbed from health care workers volunteering to this study performed on December 5, 2019. Staff were unaware that phone sampling would occur prior to the research team arriving at the ward. All clinical staff provided consent and completed an anonymous written survey about their mobile usage and habits.

In all, 30 swab samples were taken, representing 30 mobile phones, and 30 surveys were completed: five (5) from NICU, five (5) from PICU and twenty (20) from PED.

The surveys completed by healthcare staff comprised 14 questions and eight sub-questions (Appendix 1). Surveys were labelled to match the mobile phone swab label.

2.1. Sampling

Samples were taken with "Culture Swab EZ II™" (Becton Dickinson)

Table 1
Recognised human pathogen and commensal bacterial operational taxonomic units (OTUs) identified from 30 hospital staff mobile phones. Red highlight indicates 100% frequency of occurrence in that ward; orange 80–99%, and yellow 50–79%. Grey highlights ESKAPE' bacteria. NICU=Neonatal Intensive Care Unit; PED=Paediatric Emergency Department; PICU=Paediatric Intensive Care Unit (PICU).

Species	Gram	Total (n=30)	NICU (n=5)	PED (n=20)	PICU (n=5)
Micrococcus luteus	+	29	5	20	4
Staphylococcus aureus	+	28	5	19	4
Staphylococcus hominis	+	28	5	19	4
Staphylococcus epidermidis	+	27	5	17	5
Staphylococcus saprophyticus	+	23	5	16	2
Staphylococcus capitis	+	22	5	12	5
Staphylococcus haemolyticus	+	18	5	10	3
Staphylococcus warneri	+	18	4	10	4
Bacillus cereus	+	17	3	10	4
Listeria monocytogenes	+	14	1	11	2
Bacillus subtilis	+	10		8	2
Staphylococcus cohnii	+	6		3	3
Staphylococcus pasteuri	+	5		4	1
Staphylococcus xylosus	+	5		3	2
Staphylococcus lugdunensis	+	4		4	
Staphylococcus simulans	+	4	2	2	
Staphylococcus caprae	+	2		1	1
Mycobacterium abscessus	+	1		1	
Staphylococcus carnosus	+	1		1	
Staphylococcus equorum	+	1			1
Streptococcus pneumoniae	+	1			1
Acinetobacter baumannii	-	20		17	3
Pseudomonas aeruginosa	-	18	5	13	
Escherichia coli	-	9		8	1
Acinetobacter calcoaceticus	-	7		6	1
Acinetobacter calcoaceticus/baumannii complex	-	7		7	
Stenotrophomonas maltophilia	-	6		6	
Enterobacter asburiae	-	5		5	
Enterobacter cloacae	-	5		5	
Enterobacter cloacae complex	-	5		5	
Enterobacter cloacae complex 'Hoffmann cluster IV'	-	5		5	
Enterobacter_u_s	-	5		5	
Klebsiella pneumoniae	-	5		5	
Salmonella enterica	-	5		5	
Klebsiella oxytoca	-	4		4	
Pantoea septica	-	4		4	
Acinetobacter ursingii	-	3		3	
Citrobacter braakii	-	3		3	
Citrobacter freundii	-	3		3	
Enterobacter cloacae complex 'Hoffmann cluster III'	-	3		3	
Enterobacter hormaechei	-	3		3	
Enterobacter sp. BIDMC 27	-	3		3	
Enterobacteriaceae_u_s	-	3		3	
Acinetobacter lwoffii	-	2			2
Acinetobacter radioresistens	-	2		2	
Acinetobacter towneri	-	2	2		
Acinetobacter idrijaensis	-	1			1
Acinetobacter nosocomialis	-	1		1	
Acinetobacter pittii	-	1		1	
Acinetobacter schindleri	-	1			1
Bordetella pertussis	-	1		1	
Enterobacter sp. Ag1	-	1		1	
Enterobacter sp. MR1	-	1		1	
Enterococcus casseliflavus	-	1		1	
Enterococcus gallinarum	-	1		1	
Enterococcus saccharolyticus	-	1		1	
Enterococcus sp. HSIEG1	-	1		1	
Enterococcus_u_s	-	1		1	

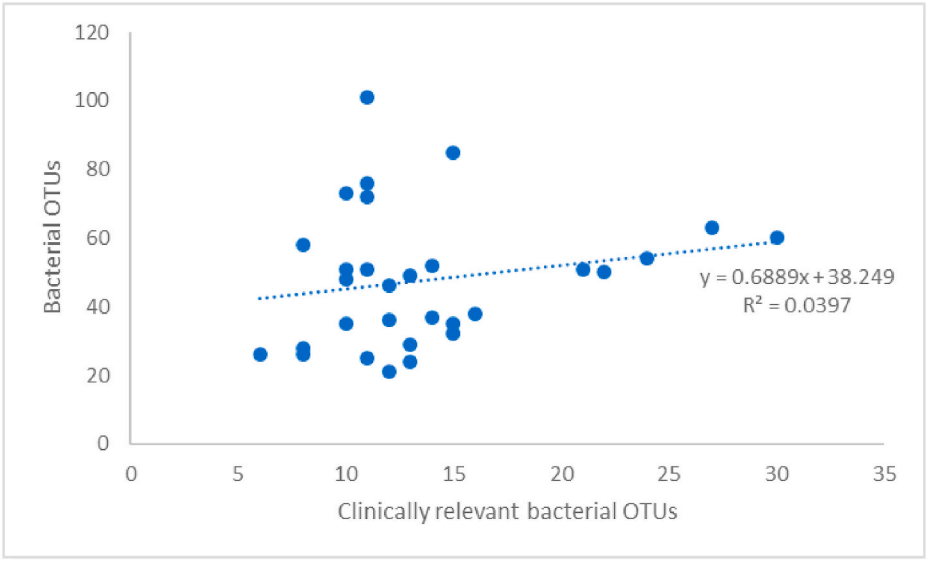

Fig. 4. Correlation between richness of all bacterial operational taxonomic units (OTUs) and recognised pathogenic and commensal OTUs for each sampled mobile phone (n = 30, p = 0.1271).

swabs. Gloves were worn when handling and swabbing the front and back of mobile phones and replaced after each swab sample to prevent cross-contamination. Following collection, the swabs were returned to the transport tube, sealed, labelled, and placed in a cooler box for transport to the laboratory.

2.2. Culture plating

The 30 individual phone swabs were removed from their transport tubes and placed each in a saline solution for 15–20 min. Each phone-derived swabbed solution was subsequently inoculated onto five different agar plates: Nutrient Agar; MacConkey Agar; Bile Esculin Agar; Horse Blood Agar; and Mannitol Salt Agar. Following incubation for 48 h, all colonies grown from the same phone were pooled for DNA extraction.

2.3. DNA extraction

Agar plates were swabbed, and DNA was extracted with a preliminary step of bead beating using 0.1 mm diameter glass beads (Bio-Spec Products #11079101) on the Powerlyser 24 homogenizer (Mo-Bio #13155). The sample was transferred to a bead tube and 800 μl of Bead Solution (Qiagen #12855-100-BS) was added. The sample was bead-beaten for 5 min at 2000 RPM, then centrifuged for 1 min at 10,000 g. Then, 60 μl of solution C1 (cell lysis buffer) was added to the sample tube and vortexed to mix. The tubes were heated at 65 °C for 10 min while mixing at 1000 RPM. Sample tubes were then vortexed for 30 s before storing overnight at −20 °C. Sample tubes were thawed at room temperature; vortexed to mix and then centrifuged for 1 min at 10,000 g. The resulting lysate was transferred to a new collection tube. DNA extraction was as per DNeasy Powersoil Kit (Qiagen #12888–100) with a final elution volume of 50 μl (sterile elution buffer EDTA free).

2.4. Metagenomic sequencing and bioinformatics analysis

Sequencing of the samples was performed at the Australian Centre for Ecogenomics, University of Queensland. Library preparation of the microbial DNA sampled were undertaken using Nextera DNA Flex Library Prep Kit (Illumina) and both quality controlled and quantified with subsequent normalisation. Multiplex pooling of library samples was undertaken prior to running in the NextSeq 500 sequencer (Illumina) on a 2 × 150 bp run with coverage of 1 Gbp per sample. Data output following the sequencing was produced as demultiplexed FASTQ files.

Following the sequencing runs, data provided as demultiplexed FASTQ files were uploaded into CosmosID (https://www.cosmosid.com/) software to identify bacteria, VFGs and ARGs. The CosmosID bioinformatics software package utilises a high-performance data-mining K-mer based algorithm that disambiguates hundreds of millions of short reads of a metagenomic sample into the discrete microorganisms engendering the particular sequences. Similarly, the collection of VFGs and ARGs in the microbiome was also identified against curated VFGs and ARGs in the databases. The overall database is derived from curated GenBook® Databases comprising over 150,000 bacteria, viruses, fungi, and protists genomes and gene sequences from both private and public sources such as NCBI- RefSeq/WGS/SRA/nr, PATRIC, M5NR, IMG, ENA, DDBJ. Data were filtered using a multi-kingdom resolutive taxonomic identification analysis built into CosmosID. This filtering was based on internal statistical scores from CosmosID, which enabled listing of results without further validation to determine their presence in the sample. Datasets were reported in two ways: (1) data were included as operational taxonomic units (OTUs) or gene Ids; and (2) selected medically relevant bacteria, bacteriophages, VFGs, and ARGs were reported in this study.

2.5. Analyses

OTUs and genes were not subject to quantitative testing. Sub-analysis included richness (total number of individual OTUs or genes), and cumulative hits (count of OTUs from all phones without removal of replicate OTUs; that is, if an OTU was found in 20 phones, it counts as one unit for richness and 20 cumulative hits).

Data were analysed for bacterial OTU richness against the following demographics: ward, gender, clinical profession, age group, type of phones sampled (mobile with buttons, smartphone, hospital phone), time of last cleaning (never, this year, this month, this week, today), phone use in toilet, current illness symptoms, and type of phone cover (none, plastic, glass). Results are not normally distributed, and were presented as simple descriptive statistics (medians, minimum and maximum values) and when tested for significant differences, this was done through analysis of variance with calculated P values followed by a Tukey Honestly Significant Difference (HSD) test in the open-access software R (cran.org) using the 'agricolae' package. Boxplots were created in the same software with the 'lattice' package. When statistical

Table 2
Matrix of ESKAPE bacteria, and closely related operational taxonomic units (OTUs; marked with ^) found on each sampled phone.

ESKAPE bacteria and closely related OTUs	Total (n=30)	PICU (n=5)					NICU (n=5)					PED (n=20)																			
		#1	#2	#3	#4	#5	#1	#2	#3	#4	#5	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12	#13	#14	#15	#16	#17	#18	#19	#20
^*Enterococcus casseliflavus*	1																														x
^*Enterococcus gallinarum*	1																							x							
^*Enterococcus saccharolyticus*	1																							x							
^Enterococcus sp. HSIEG1	1																							x							
^*Enterococcus*_u_s	1																														x
Staphylococcus aureus	28	x		x	x	x	x	x	x	x		x	x	x			x	x	x	x	x	x	x	x	x	x	x	x	x	x
Klebsiella pneumoniae	5																	x					x		x	x				x
Acinetobacter baumannii	20		x		x	x							x	x	x		x	x	x	x	x	x	x	x	x	x	x		x	x
^*Acinetobacter calcoaceticus/baumannii* complex	7																x		x	x			x		x		x		x	x
Pseudomonas aeruginosa	18						x	x	x	x	x	x	x		x	x	x	x	x			x	x		x	x	x	x		x
Enterobacter asburiae	5						x										x						x		x	x				x
Enterobacter cloacae	5																x						x		x	x				x
Enterobacter cloacae complex	5																x						x		x	x				x
Enterobacter cloacae complex 'Hoffmann cluster IV'	5																x						x		x	x				x
*Enterobacter*_u_s	5																x						x		x	x				x
Enterobacter cloacae complex 'Hoffmann cluster III'	3																								x	x				x
Enterobacter hormaechei	3																								x	x				x
Enterobacter sp. BIDMC 27	3																								x	x				x
Enterobacteriaceae_u_s	3																								x	x				x
Total OTUs in each phone	–	1	1	1	2	2	2	2	2	2	2	2	3	2	2	2	2	10	3	4	2	2	3	10	5	14	13	3	2	3	16

analyses were not possible due to limited sample size, qualitative interpretation of the answers was undertaken.

2.6. Ethics and funding

The Gold Coast Health (GC HREA 46569) and Bond University Human Research Ethics Committees (16,004) approved the study.

Funding for the DNA sequencing and Laboratory Support was made available through Bond University.

3. Results

All phones sampled (n = 30) were contaminated with bacteria and showed growth on agar plates (Fig. 1). The average total sequencing reads across all 30 sequenced samples was 7.477 million reads per phone, and the range was from 4.448 to 17.659 million reads (Fig. 2).

3.1. Bacterial metagenomic sequencing

A total of 399 bacterial OTUs were identified from the 30 phones, with 1432 cumulative hits. The bacteria richness had a median of 48.5 per phone (range 21–101) and was significantly different between phones from NICU and PICU staff (Fig. 3A).

Among the 399 bacteria identified in this study, 58 recognised human pathogen and commensal bacterial OTUs were present and of these, 11 were isolated from more than half the mobile phones sampled (Table 1). Mobile phones had a median of 12 recognised human pathogen and commensal bacteria each (ranging from 6 to 30). There was a weak correlation, not statistically significant, between all bacterial OTUs and clinically relevant OTUs found in each phone ($R^2 = 0.0397$; $p = 0.1271$; Fig. 4).

Of the 58 recognised human pathogen and commensal bacteria, 37 were Gram-negative and 21 were Gram-positive (Table 1). One Gram-positive bacterial species from the PED was acid-fast. Gram-positive bacteria were evenly present across all three wards, with no observable differences between wards. Gram-negative bacteria were more frequent in mobile phones from staff in the PED. Of the 37 OTUs of Gram-negative recognised human pathogen and commensal bacteria identified, 33 (133 cumulative hits) were detected from PED, whereas 2 (7 hits) were detected from NICU, and 6 (9 hits) were detected from PICU.

Some colonisation patterns noted were: (1) all *Enterobacter* OTUs were found on five phones in PED; (2) *Enterococcus* OTUs were on two PED phones; (3) *Pseudomonas aeruginosa* was not found in PICU while prevalent in the two other wards and on all phones tested in NICU; (4) three species of *Acinetobacter* (*A. lwofii, A. idrijaensis, A. schindleri)* were only found in PICU, all three on a single phone and *A. lwofii* on another phone; (5) *A. towneri* was only found on two phones in the NICU; (6) notably, NICU only had two Gram-negative species of bacteria detected, *A. towneri* and *P. aeruginosa*.

Six coagulase-negative *Staphylococci* (CoNS) species were present on 18 of the 30 phones sampled (Table 1), and these were prevalent in all three wards: *Staphylococcus hominis, S. epidermidis, S. saprophyticus, S. capitis, S. haemolyticus*, and *S. warneri*. At least 80% of phones in each ward harboured at least three of these six CoNS species. In all, 24 CoNS OTUs were identified in this study with 213 cumulative hits.

Escherichia coli was found on nine mobile phones; one from PICU and eight from PED. Other notable findings were *Salmonella enterica* (5 phones from PED), which is transmitted mainly through faeces and contaminated water; *Listeria monocytogenes* (11 phones from PED, 2 PICU, 1 NICU), which can be fatal for immunocompromised individuals, including pregnant women, elderly, and neonates; *Bordetella pertussis* (1 PED phone) and *B. bronchiseptica* (2 PED and 2 PICU phones); and the emerging global pathogens *Brevundimonas aveniformis* (2 PED, 5 NICU phones), *B. diminuta* (8 PED phones), and *B. naejangsanensis* (5 PED phones). *Stenotrophomnas maltophilia*, known to be associated with HAIs and chronic pulmonary disorders, such as cystic fibrosis, was isolated

Table 3
Number of Virulence Factor Genes (VFGs) and cumulative hits (Hits) associated with bacterial species or operational taxonomic units.

Operating Taxonomic Units	VFGs	Hits	Operating Taxonomic Units	VFGs	Hits	Operating Taxonomic Units	VFGs	Hits
Staphylococcus aureus	173	633	*Proteus mirabilis*	2	18	*Bacillus* 65	1	3
Enterobacter aerogenes	57	170	*Salmonella Infantis*	2	14	*Bacillus* 82	1	3
Bacillus anthracis	18	55	*Enterococcus faecalis*	2	9	*Bacillus* 88	1	2
Bacillus cereus	18	42	*Streptococcus pyogenes*	2	4	*Bacillus* 116	1	2
Klebsiella pneumoniae	9	45	*Enterococcus gallinarum*	2	2	*Citrobacter freundii*	1	2
Pseudomonas aeruginosa	8	30	*Staphylococcus epidermidis*	1	21	*Enterococcus hirae*	1	2
Staphylococcus lentus	7	66	*Vibrio cholerae*	1	8	*Bacillus* 76	1	1
Escherichia coli	7	25	*Bacillus subtilis*	1	6	*Bacillus* 91	1	1
Klebsiella oxytoca	5	20	*Bacillus* 85	1	5	*Bacillus* 104	1	1
Enterococcus faecium	5	10	*Pseudomonas putida*	1	5	*Bacillus* 107	1	1
Serratia marcescens	3	17	*Bacillus* 113	1	4	*Bacillus* 110	1	1
Shigella flexneri	3	12	*Bacillus* 94	1	4	*Bacillus* 119	1	1
Salmonella typhimurium	3	8	*Salmonella* GENE	1	4	*Morganella morganii*	1	1

Table 4
Antibiotic Resistant Genes (ARGs) groups isolated, their richness and cumulative hits (Hits).

Antibiotic Resistance Genes (ARGs)	Richness	Hits	Antibiotic Resistance Genes (ARGs)	Richness	Hits
Beta-lactam	34	107	AR 272,645 2429 Branch	1	3
Aminoglycoside	26	73	arlR	1	3
MDR-Efflux-pump, Efflux-pump, MDR-Efflux-complex	18	97	Repressor-of-MepA mepR	1	3
Macrolide	16	88	Response-regulator arlS	1	3
Tetracycline	5	20	Sensor-protein smeS	1	3
Quinolone	5	17	AR 277,676 2398 Branch	1	2
Trimethoprim	3	22	bleomycin resistance protein BRP	1	2
Phenicol	3	7	Fusidic acid fusC	1	2
Sulphonamide sul 2	1	12	Integron-mediated-quinolone-resistance qnrVC1	1	2
Signal-transcuding-protein mecR1	1	10	metallo-beta-lactamase bcII	1	2
AR 269,551 2523 Branch	1	9	mprF	1	2
Regulator mgrA	1	6	Responder smeR	1	2
Repressor-of-transcription mecI	1	6	metallo-beta-lactamase bcI	1	1
Tunicamycin-resistance tmrB	1	6	Plasmid-or-transposon-encoded-chloramphenicol-exporter cmx	1	1
Fosfomycin fosA 2192 Branch	1	4			
MDR-transporter emrD	1	4	Vancomycin vanXYC	1	1

from six phones from the PED. Finally, *Streptococcus pneumoniae*, which is an upper airway commensal, but can cause otitis media and sinusitis, and more severe infections, such as community-acquired pneumonia and meningitis was found on one PICU phone.

Various 'ESKAPE' pathogens (*E. faecium, S. aureus, Klebsiella pneumoniae, A. baumannii, P. aeruginosa,* and *Enterobacter* spp.), commonly associated with increasing virulence and multi-antibiotic resistance, were found in this study (Table 2). Despite not detecting *E. faecium,* five other species of *Enterococcus* were identified.

At least one bacterium from the ESKAPE group was found on all phones sampled (Table 2). Five phones from PED (n = 20) contained at least one OTU from each of the five ESKAPE bacteria. PICU phones (n = 5) contained one or two ESKAPE bacteria (*S. aureus* and/or *A. baumannii)*. All NICU phones contained two ESKAPE bacteria (*S. aureus* and *P. aeruginosa*). Detections of ESKAPE OTUs ranged from 2 to 16 for PED samples.

3.2. Virulence factor, antibiotic resistance genes and bacteriophage metagenomic sequencing

3.2.1. Virulence factor genes

The total number of VFGs detected was 347, and the cumulative hits were 1258. Sampled phones had median richness of VFGs of 29 (Fig. 3B), ranging from 11 to 169 per phone.

The 23 most frequently occurring VFGs were found on at least 10 of the 30 mobile phones sampled. These were most commonly genes from *S. aureus* (15 genes, 282 cumulative hits), *S. lentus* (4 genes, 47 hits – all within the PED), and *S. epidermidis, Serratia marcescens, K. pneumoniae,* and *P. aeruginosa* (1 gene each, with 21; 12; 12; and 10 hits respectively). Alternatively, 237 VFGs were found on three or fewer mobile phones, of which 98 were found on a single mobile phone sampled.

All VFGs were from 39 bacteria species or OTUs, *S. aureus* (173 VFGs, 633 cumulative hits), *E. aerogenes* (57 VFGs, 170 hits), *Bacillus anthracis* (18 VFGs, 55 hits) and *B. cereus* (18 VFGs, 42 hits). All other OTUs had less than 10 VFGs detected (Table 3).

3.2.2. Antibiotic resistance genes

ARGs were detected on all phones sampled with median of 17.5 ARGs per phone (range from 6 to 41) (Fig. 3C). There were 133 ARGs detected, with a cumulative total of 520 hits. The most common classes of ARGs encoded resistance to beta-lactam, aminoglycoside and macrolide antibiotics and upregulated efflux pumps (Table 4). There was a significant difference between number of ARGs per phone between NICU and the other two wards, whereas PICU and PED were not significantly different from each other (Fig. 3C).

Overall, 155 bacteriophages or bacteriophage OTUs were detected in 734 cumulative hits. The median bacteriophage richness per phone was 21.0 (Fig. 3D). The number of bacteriophages isolated from phones ranged from 4 to 63. Only 16 bacteriophages were detected on 10 or more phones, whereas 84 were detected on three or fewer phones. Bacteriophages and viruses specific to *Staphylococcus* were the most common followed by *Salmonella* (Table 5).

3.3. Clinical staff attributes

The results from the questionnaire (Appendix 1) were summarised and compiled in Table 6.

None of the 30 participating staff had travelled overseas in the 4 weeks prior to sampling; no staff were taking antibiotics; all staff reported washing their hands with water and soap after using the toilet; and all staff believed their phones were contaminated.

Despite all staff believing their phones were contaminated, only 10 of

Table 5
Number of bacteriophages and cumulative hits (Hits) associated with genera of bacteria.

Target genus	Phages	Hits	Target genus	Phages	Hits	Target genus	Phages	Hits
Staphylococcus	55	383	*Phietavirus*	1	9	*Streptococcus*	1	1
Salmonella	15	59	*Siphoviridae*	1	8	*Microbacterium*	1	1
Bacillus	13	49	*Enterobacterial*	1	5	*Lederbergvirus*	1	1
Escherichia	13	45	*Shigella*	1	5	*Likavirus*	1	1
Pseudomonas	10	35	*Myoviridae*	1	3	*Pectobacterium*	1	1
Enterobacteria	9	35	*Hendrixvirus*	1	3	*Mycobacterium*	1	1
Stenotrophomonas	6	18	*Biseptimavirus*	1	2	Stx2-converting	1	4
Acinetobacter	3	36	*Lambdavirus*	1	2	Viruses	1	2
Propionibacterium	3	3	*Triavirus*	1	2	Wbetavirus	1	2
Erwinia	2	5	*Vibrio*	1	2	Pamx74virus	1	1
Cronobacter	2	5	*Psychrobacter*	1	2	uncultured	1	1
Rhizobium	2	2						

30 respondents indicated they had ever cleaned their phones. Five staff cleaned their phones with lint felt cloth and five with alcohol wipes. Of the five staff who disinfected their phones with alcohol wipes, one had done so that day, one within a week, two within a month and one within a year. The sample size of staff who cleaned their phone was deemed insufficient to analyse results between groups.

When analysed as a whole, phones that had at some time been cleaned by any method did not show bacterial OTU richness difference from the group that had never cleaned their phones (Fig. 5).

4. Discussion

Australia has limited surveillance and reporting of HAIs, which are published on the MyHospitals website [33]. A HAI prevalence study was performed in 1984, with a second limited study in 2018, showing that on any given day, 10% of acute adult inpatients have at least one HAI. Understanding the role mobile phones might play in contributing to HAIs would appear to be an important research question for our health system. HAIs and antibiotic resistance disproportionally affect the most vulnerable in our community. This research has shown that high rates of viable pathogens and resistance genes can be present on mobile phones in clinical settings caring for these vulnerable patients.

Strategies to reduce infection within healthcare settings, such as hand washing, were implemented in the 19th century based on the pioneering physician Ignaz Semmelweis who identified and then emphasised the importance of hand washing [34]. Indeed, hand hygiene has proven effective in slowing transmission of human pathogens for more than a century. The finding of a large number of potentially very serious pathogens on the surface of health care workers' mobile phones highlights the need for stricter hygiene requirements for clinical practice in hospitals and in the broader community today.

This research has demonstrated that viable pathogenic bacteria are ubiquitous on health care workers' mobile phones within a hospital setting. Of the 399 viable bacterial OTUs detected, 58 were identified as human pathogens or commensals. The remaining 341 OTUs are still of interest as they demonstrate the microbial density contaminating mobile phones and the possibility for non-human pathogens to be present and represent the possibility that phones could act as a platform for microbial reproduction. These organisms may also act as reservoirs for VFGs and ARGs that can be transferred to human pathogens.

Eleven of the human pathogen and commensal bacteria identified, *S. aureus, S. hominis*, *S. epidermidis, S. saprophyticus, S. capitis, S. haemolyticus, S. warneri, A. baumannii, Micrococcus luteus, B. cereus,* and *P. aeruginosa*, were found in high numbers on the sampled phones and are recognised as causative agents for severe or life-threatening complications in immunocompromised people, in particular, intensive care patients. Specifically, *S. epidermidis*, found on 17 of the 30 sampled phones, has been recognised as the most common cause of late-onset sepsis in neonatal intensive care units [35].

ESKAPE pathogens (*E. faecium, S. aureus, K. pneumoniae, A. baumannii, P. aeruginosa and Enterobacter* species) can be MROs and a leading cause of worldwide HAIs [36]. An unexpected result from our research was detecting ESKAPE bacteria on all mobile phones sampled. Between one and 16 OTUs of ESKAPE bacteria were found on each sampled phone. Despite lacking direct evidence that these pathogens had been transferred from mobile phones to patients, they were all viable and should be considered a potential source of infection.

Further to bacterial OTUs being present on the surface of phones, DNA evidence of a wide range of ARGs and bacteriophages was identified following DNA sequencing. Previous studies confirm antibiotic resistant organisms on the surface of mobile phones belonging to hospital inpatients [37] and healthcare staff [38]. The latter study revealed mobile phones were enriched with the pathogens found on the fingers of hospital staff. Our team hypothesises that mobile phones harbouring a high density of microbes could facilitate horizontal gene transfer of ARGs between and within bacterial species leading to the generation and spread of antimicrobial resistant strains. This hypothesis is supported by earlier research where viable pathogenic bacteria were found to persist for weeks on touch surfaces and plasma-mediated horizontal gene transfer of ARGs was observed [39].

Our study has shown that the most prevalent classes of ARGs found on the 30 sampled phones encoded resistance to beta-lactam, aminoglycoside and macrolide antibiotics, and efflux pumps. Given the presence of a high number of ARGs and of ESKAPE bacteria on the surface of mobile phones, these fomites are considered a possible transmission pathway for pathogen movement within hospitals, in the community and globally.

This study was not designed to prove if microbes on mobile phones cause HAIs in patients. Nevertheless, HAIs pose a major worldwide public health threat along with MROs as leading causes for morbidity and mortality. In developing and developed countries, 10% and 7% hospitalised individuals contract a HAI, respectively [1]. Antimicrobial resistance represents ongoing therapeutic challenges, and cross-infection by MROs in hospitals has led to uncertainty as to how these pathogens will be managed with limited treatment options remaining. The presence of viable antimicrobial resistant organisms on mobile phones in hospital settings could add substantially to the challenge of managing infections by these agents.

Employing a swab-culture-next generation sequencing method followed by OTU identification using gene libraries allowed us to identify a much larger number of species and OTUs than previous studies [15]. However, the results presented here are likely an underestimate of the total microbial burden on mobile phones. The methods used in this study were limited to five different types of agar, which differentially allowed species of microbes to be cultured. The results presented here are also limited to bacteria and bacteriophages. More inclusive results are logically expected from a broader range of agar, which would enable culture of more species of bacteria, fungi and other organisms. A much longer list of results is also expected from direct swab-to-NGS; which is a methodology that enables the detection of microbes including animal and plant viruses and other micro-organisms that are not culturable, but has the drawback of detecting DNA and RNA material from both viable

Table 6
Results from questionnaires split into variables and groups and tabulated against bacterial operational taxonomic unit (OTU) richness average, standard deviation (SD), minimum (min), maximum (max) and total OTUs for each group. Results for each variable were submitted to an Honestly Significant Difference (HSD) test and found to be not statistically different in all cases except ward. PICU=Paediatric Intensive Care Unit; NICU=Neonatal Intensive Care Unit; PED= Paediatric Emergency Department.

Variable and groups	**sample size**	**median**	**minimum**	**maximum**	**OTUs**
Total	30	48.5	21	101	399
Ward	**sample size**	**median**	**minimum**	**maximum**	**OTUs**
PICU	5	29.0	25	35	88
NICU	5	73.0	48	101	143
PED	20	49.5	21	85	312
Gender	**sample size**	**median**	**minimum**	**maximum**	
Female	19	50.0	24	101	
Male	7	58.0	25	72	
Undisclosed	4	41.5	21	49	
Age	**sample size**	**median**	**minimum**	**maximum**	
18–25	7	51.0	21	101	
26–55	17	38.0	24	85	
>55	6	61.5	25	76	
Profession	**sample size**	**median**	**minimum**	**maximum**	
Doctor	8	48.5	21	76	
Medical student	2	27.5	26	29	
Ward Nurse	19	50.0	24	101	
Ward pharmacist	1	35.0	n/a	n/a	
Ever cleaned phone? When?	**sample size**	**median**	**minimum**	**maximum**	
No	20	49.5	21	85	
today	1	48.0	n/a	n/a	
this week	4	43.5	29	76	
this month	3	35.0	26	73	
this year	2	63.0	25	101	
Type of phone	**sample size**	**median**	**minimum**	**maximum**	
Hospital	3	38.0	21	46	
Mobile phone small screen	2	60.5	48	73	
Smartphone large screen	25	50.0	24	101	
Use phone in toilet?	**sample size**	**median**	**minimum**	**maximum**	
No	6	34.0	24	51	
Yes	24	50.5	21	101	
Suffering from infection?	**sample size**	**median**	**minimum**	**maximum**	
No	24	50.0	21	101	
Yes, mild infection, no antibiotics	6	41.5	25	72	
Screen cover?	**sample size**	**median**	**minimum**	**maximum**	
No	12	43.0	26	101	
Yes	18	50.0	21	85	
Yes, glass (subset)	6	48.5	28	72	
Yes, plastic (subset)	11	51.0	21	85	

and not viable organisms.

Additional limitations are that the study involved only a small number of staff and their mobile phones from a single centre. Although the results may not be generalisable to other centres or populations, the staff came from three distinct services within a hospital setting. It is of interest that the service that interacts mostly with the community, the PED, had mobile phones with the greatest prevalence of environmental organisms, while the phones from staff attending the two intensive care units were populated more by human pathogens and commensals. Whether this represents more environmental cleaning and/or placement of phones in these settings was not explored in our study. Finally, as this study was conducted in a paediatric setting, mobile phones of patients were not tested, but this would be of an interest in adult wards.

Mobile phones have become omnipresent in life, including in healthcare settings, and hygiene practices solely focused on handwashing are likely insufficient if no action is taken to disinfect phones. It is logical to infer that cross-contamination between phones and hands would occur, since the average person uses their phones for 3.5 h each day [40].

This research identified that from a sample of 30 health care workers, the majority use their mobile phones in bathrooms, and despite washing their hands with water and soap they do not regularly, if ever, clean their phones. The Neonatal Intensive Care Unit had a poster (Fig. 6) over the entry handwash station requesting phones to be wiped, and yet, through the questionnaire, it was found only one of the NICU staff phones was cleaned with alcohol wipes (the remaining 4 had been cleaned with lint felt cloth). No similar signs were present in the other two wards sampled in this study.

While medical practitioners need to be more conscious of effective mobile phone disinfection, it is also important that the general community and in particular those that undertake self-medication, such as catherization, understand the importance of cleaning mobiles phones and other touch screen devices. New materials such as copper coated phone cases or plastic films that prevent microorganism adhesion need to be explored as future infection control mechanisms. Simultaneously (and in particular, until better technologies are available and implemented), phone decontamination should be promoted to all users. We also hypothesise that the microbial pathogens healthcare workers are exposed to in their professional setting may be introduced into the community via the mobile phone pathway. This hypothesis needs further research and should be considered a priority in light of the current global pandemic.

Finally, the efficacy of decontamination procedures for mobile phones and other touchscreen devices should be elucidated, taking into account different materials and procedures (such as Ultraviolet radiation); and a systematic and widespread disinfection protocol in medical settings to prevent cross-contamination between phones and hands should be developed and implemented on a large scale. Additionally, and until further research is conducted confirming whether mobile phones are important fomites in transmitting infections in healthcare and community settings, we suggest that as an extra simple intervention mobile phones are added to the 'five moments of hand hygiene'.

In conclusion, we provide further evidence that pathogens and microbes in general are present in commonly used mobile phones and smartphone devices. From patients, food handlers, healthcare staff, travellers (planes, boat cruises) to conferences attendees of national and international seminars), highly touched devices like mobile phones are constantly enriched by microbiota and pathogenic microbes. However, mobile phones are poorly known as contaminated platforms and often ignored for their mean of potential microbial transmission; Mobile phones are "Trojan horses" [15] and the challenge in preventing disease

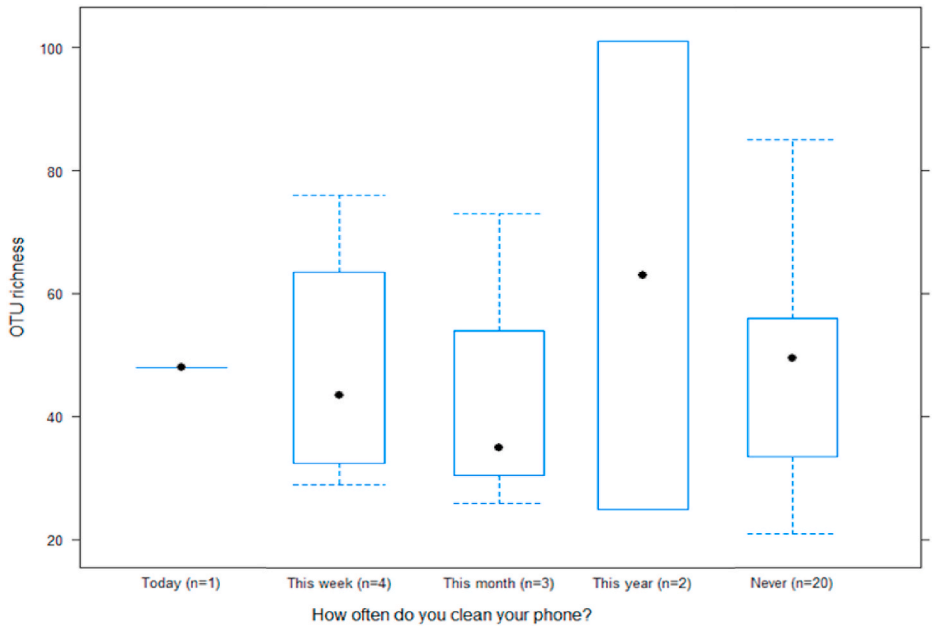

Fig. 5. Bacterial operational taxonomic unit (OTU) richness against frequency in which hospital staff reported cleaning their phones. Half of the respondents who cleaned their phones did so with a lint felt cloth and half with alcohol wipes.

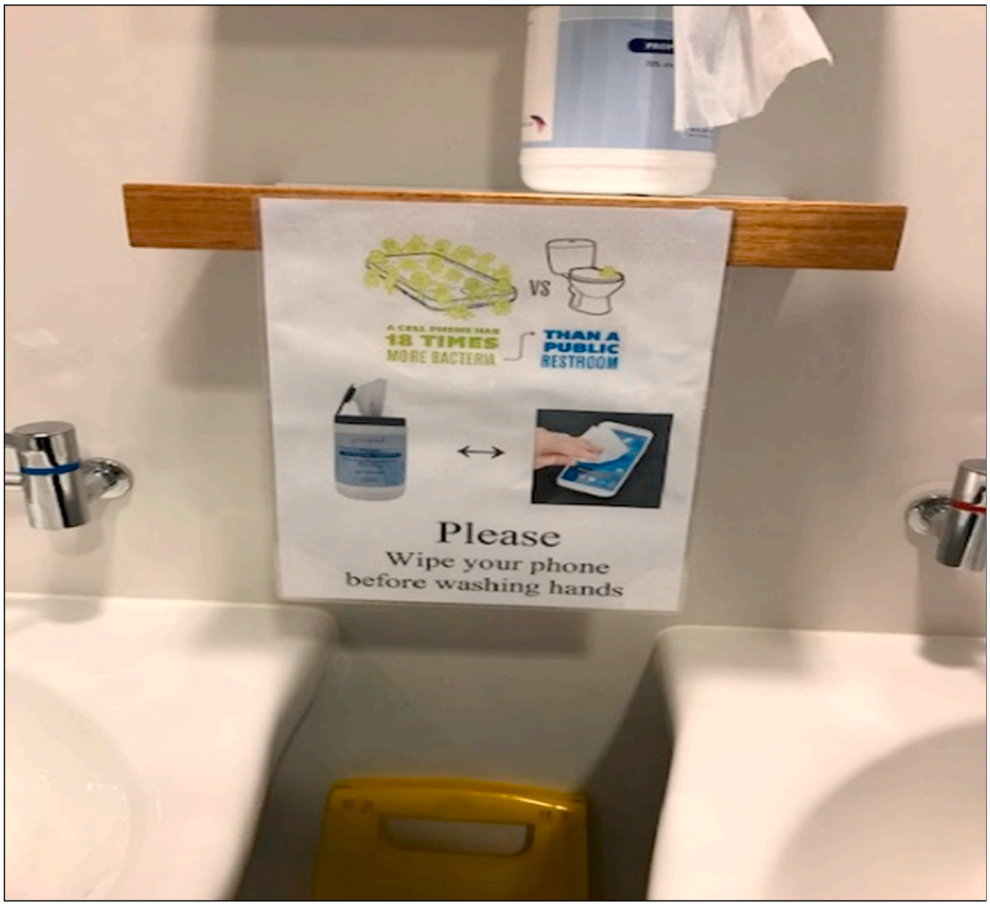

Fig. 6. Poster found by the sinks at the staff entrance of the Neonatal Intensive Care Unit (NICU) ward on the day of sampling.

spread resides in recognising fomites in general are possibly contributors to outbreaks and epidemics. As an example, RNA of SARS-Cov-2 virus responsible for COVID-19 has been found on mobile phones [20].

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CRediT authorship contribution statement

Lotti Tajouri: Conceptualization, Methodology, Data curation, Writing – original draft. **Mariana Campos:** Data curation, Writing – original draft. **Matthew Olsen:** Conceptualization, Methodology, Data curation, Writing – original draft. **Anna Lohning:** Writing – review & editing. **Peter Jones:** Writing – original draft, Writing – review & editing. **Susan Moloney:** Writing – review & editing. **Keith Grimwood:** Writing – review & editing. **Hassan Ugail:** Data curation, Writing – review & editing. **Bassam Mahboub:** Writing – review & editing. **Hamad Alawar:** Writing – review & editing. **Simon McKirdy:** Conceptualization, Methodology, Writing – review & editing. **Rashed Alghafri:** Conceptualization, Methodology, Writing – original draft.

Declaration of competing interest

NONE.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tmaid.2021.102095.

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